

ART 34 AMDE

48

CLAIMS:

1. A method of converting a first composition comprising ferulic acid into a second composition comprising vanillin, said method comprising treating said first composition with *Pseudomonas putida* under conditions such that ferulic acid is converted into vanillin, and the vanillin accumulates.
2. A method according to claim 1 wherein said strain is *Pseudomonas putida* IMI382568.
3. A method according to claim 1 or 2 wherein said strain is capable of producing both vanillic acid and vanillin from ferulic acid, the ratio thereof being pH-dependant; and wherein a pH is selected and maintained which relatively favours accumulation of vanillin.
4. A method of converting a first composition comprising ferulic acid into a composition comprising vanillin by (i) converting said first composition which comprises ferulic acid into a second composition which comprises vanillic acid by treatment with a microorganism selected from *Pseudomonas putida* and *Rhodotorula glutinis*; and (ii) treating said second composition with a second microorganism selected from *Aspergillus fumigatus* and *Micromucor isabellinus* for converting vanillic acid with vanillin.
5. A method according to claim 4 wherein said first microorganism is *Pseudomonas putida* NCIMB40988 or *Rhodotorula glutinis* IMI379896.

A4
Sub

6. A method according to any preceding claim including a preliminary step of obtaining said first composition comprising ferulic acid from a plant material by a process comprising:

- 5 (a) treating the plant material to produce a solution containing a ferulic acid ester; and
- (b) treating said solution with an enzyme composition having ferulic acid esterase activity under conditions such that ferulic acid esters are converted
- 10 into ferulic acid.

7. A method according to claim 6 wherein said plant material is selected from maize, wheat, sugar beet and rice materials.

15

12
8. A method according to claim 7 wherein said plant material comprises fibre, bran or straw.

A4
Sub

20

9. A method according to claim 6, 7 or 8 wherein in step (a) the plant material is treated with a solution containing citric acid.

25

10. A method according to claim 9 wherein said plant material is treated in the temperature range 50-250°.

11. A method according to claim 6 wherein the plant material comprises sugar beet fibre and step (a) involves heating in water.

30A4
Sub

12. A method according to any of claims 6 to 11 wherein step (b) employs an enzyme derived from a species of *Aspergillus* or *Humicola insolens*.

13. A method according to claim 10 wherein the enzyme is derived from *Humicola insolens* and treatment is effected substantially in the pH range 6-7.

14. A method of converting a first composition comprising a ferulic acid ester into a second composition comprising vanillic acid, said method comprising treating said first composition with one or more microorganisms selected from *Penicillium* and *Aspergillus* species possessing both ferulic acid esterase activity and intrasidechain cleavage activity such that they are capable of converting ferulic acid glycosides into vanillic acid under conditions such that said ester is converted into vanillic acid.

15. A method according to claim 14 wherein said microorganism is selected from *P.chrysogenum*, *A.niger* and *A.flavus*.

16. A method according to claim 15 wherein said microorganism is selected from *P.chrysogenum* IMI379900, *A.flavus* IMI379895 and *A.niger* IMI379904.

17. A process according to any of claims 14 to 16 wherein said ferulic acid ester is provided in the form of plant material, said microorganism acting directly on said plant material.

18. A method according to any of claims 14 to 17 wherein said second composition is treated with one or more further microorganisms for converting said vanillic acid into vanillin.

19. A method according to claim 18 wherein said second composition is treated with *Aspergillus fumigatus* or *Micromucor isabellinus* for converting vanillic acid into vanillin.

5

20. A method according to claim 19 wherein said second composition is treated with *A.fumigatus* IMI379902 or *M.isabellinus* IMI379893.

10
Sub

21. A method according to any of claims 1 to 13 or 18 to 20 wherein said conversion into vanillin is effected in an aqueous phase which is contacted with an organic phase which extracts said at least one desired component.

15

22. A method according to claim 1 or claim 4 including a preliminary step of obtaining a strain of microorganism for use in the method of claim 1 or step (ii) of claim 4 comprising screening a multiplicity of colonies by means of a reagent suitable for detecting aldehydes.

20

23. A method according to claim 22 wherein the multiplicity of colonies are obtained by mutation.

25

24. *Pseudomonas putida* NCIMB40988 or a mutant thereof capable of converting ferulic acid into vanillic acid.

25. *Rhodotorula glutinis* IMI379896 or a mutant thereof capable of converting ferulic acid into vanillic acid.

30

26. *Penicillium chrysogenum* IMI379900 or a mutant thereof capable of converting a ferulic acid ester into vanillic acid.

RECEIVED 1980-05-13

27. *Aspergillus flavus* IMI379895 or a mutant thereof capable of converting a ferulic acid ester into vanillic acid.
- 5 28. *Aspergillus niger* IMI379904 or a mutant thereof capable of converting a ferulic acid ester into vanillic acid.
- 21 29. *Pseudomonas putida* IMI382568 or a mutant thereof
10 capable of converting ferulic acid into vanillin.
30. *Aspergillus fumigatus* IMI379902 or a mutant thereof capable of converting vanillic acid into vanillin.
- 15 31. *Micromucor isabellinus* IMI379893 or a mutant thereof capable of converting vanillic acid into vanillin.
- 34
Sub 32. A genetically engineered organism which has been transformed with nucleic acid derived directly or
20 indirectly from the strain defined in any of claims 24 to 31 and has thereby acquired the capability set out in that claim.
- 25 33. An extract or enzyme(s) of an organism defined in any of claims 24 to 32 possessing the activity specified for the organism.
- 110
add
- W. J. B. 10/10/10
W. J. B. 10/10/10